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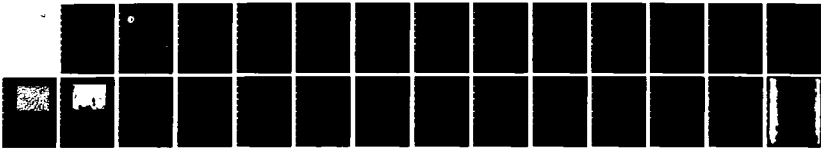

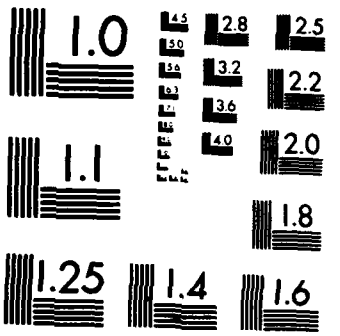

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AFRRI SCIENTIFIC REPORT

The toxicity of petroleum and shale JP5

V. Bogo
R. W. Young
T. A. Hill
C. L. Feser
J. Nold
G. A. Parker
R. M. Cartledge

DEFENSE NUCLEAR AGENCY
ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
BETHESDA, MARYLAND 20814

REVIEWED AND APPROVED

WALTER F. BURGHARDT, Jr.
Capt, USAF, BSC
Executive Officer
Behavioral Sciences Department

LAWRENCE S. MYERS, Ph.D.
Scientific Director

BOBBY R. ADCOCK
COL, MS, USA
Director

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The toxicity of petroleum- and shale-derived jet propulsion fuel #5 (JP5) was evaluated in a series of acute gavage and subchronic inhalation studies with rats. In the gavage studies, the $LD_{50/14}$ for rats was 26 ml/kg for Gary-Western shale, 39 ml/kg for Sohio shale and greater than 60 ml/kg for Exxon shale and petroleum JP5. Significant hepatic periportal fatty degeneration and renal eosinophilic hyaline droplets were observed for all fuels. Multiple hepatic cytoplasmic vacuoles were detected as early as 6 hours after

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*from the Naval Medical Research Institute, Bethesda, Maryland

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both petroleum and Sohio shale JP5 exposures but were undetectable after 96 hours. Weight and consumption of food and water were reduced for 2 to 3 days after administration of petroleum or Sohio shale JP5. Activity markedly increased between 2.5 and 6 hours after dosing for both petroleum and Sohio shale JP5.

The inhalation studies showed that water consumption increased after 8 days of exposure to petroleum or Sohio shale and remained elevated for the duration of the 30-day studies. However, no significant effects on tissue morphology or hepatic and renal serum chemistries were observed after exposure to petroleum or Sohio shale JP5, and peak amplitudes or latencies for the SEPs did not significantly change during the 30-day exposure to Sohio shale JP5.

THE TOXICITY OF PETROLEUM AND SHALE JP5

V. Bogo[1], R. W. Young[1], T. A. Hill[2], C. L. Feser[1], J. Nold[1], G. A. Parker[1], and R. M. Cartledge[1]

[1]Armed Forces Radiobiology Institute and
[2]Naval Medical Research Institute
Bethesda, MD 20814

Abstract

The toxicity of petroleum- and shale-derived jet propulsion fuel #5 (JP5) was evaluated in a series of acute gavage and subchronic inhalation studies with rats. One petroleum sample and three different shale samples were used in the gavage studies. Petroleum- and Sohio-shale-derived JP5 were evaluated in the inhalation studies. Gavage LD_{50}'s were obtained for all samples. Subsequent gavage experiments at doses from 3 to 24 ml/kg were performed to test for alterations in behavior, histopathology, and serum chemistries. Inhalation studies were conducted to assess behavioral and neurophysiological toxicity at concentrations from 1100 to 1600 mg/m^3. Behavioral evaluations included measurements of home cage activity, food and water consumption, weight, aggression, motor integration, and general behavior. Neurophysiological function during inhalation exposure to Sohio JP5 was evaluated using somatosensory evoked potentials.

The most consistently observed evidence of toxicity in the gavage studies was found in the histopathology and serology. For all fuels tested, histopathological lesions were found in the liver and kidneys, and elevated serum chemistries were obtained. The most significant morphological change was periportal fatty degeneration in the liver. No differences were observed in severity of the histopathology produced by the four fuels. However, the shale fuel with the highest content caused the greatest change in serum chemistries. These differences in serum chemistries were paralleled in the LD_{50}'s. The LD_{50} was much lower for the two shales with the highest nitrogen content than for either the third shale or the petroleum, suggesting that the refining process influenced the degree of toxicity.

Behavioral changes occurred after oral administration of both petroleum and Sohio shale JP5, but these changes seemed more related to the resulting irritation than to any neurotoxicity. For example, home cage activity increased markedly whereas weight and consumption of food and water decreased. The increased activity produced by petroleum JP5 lasted from 2 to 4 hours after gavage, but a corresponding performance change did not occur in a motor integration task. Some subjects exposed to petroleum JP5 were hypersensitive to touch; a few multilated their tails, and others became aggressive. However, this increase in aggression could not be confirmed when measured at a later time with a specific-function test for this phenomenon.

During the inhalation studies of petroleum and Sohio JP5, significant increases in water consumption occurred with both fuels, beginning on the 8th day of exposure and continuing throughout the studies. This suggests that the renal toxicity of JP5 found after oral administration may also be seen after inhalation exposure, and that its occurrence is independent of product origin, refinery process, or route of administration. No other changes were observed in the other behavioral tests, the event-related potentials, or the histopathology.

This work was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency and Naval Medical Research Development and Command under Research Work Unit MJ00067. Research was conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency or Naval Medical Research Command has been given or should be inferred.

Introduction

In light of the dwindling national reserves of petroleum crude oil, the U.S. Navy has been assessing for several years the feasibility of producing synthetic fuels extracted from coal and shale deposits [1]. Current attention has centered on shale crude, which has proven to be a reasonable substitute in a number of demonstration runs. For example, JET-A, JP4, JP5, JP8, and diesel fuel marine (DFM), which met most operational specifications [2, 3], were produced in a recent demonstration run at the Sohio refinery.

Questions as to the feasibility of synthetic fuels include not only their operational suitability, but also their potential hazard to personnel in the work place. Therefore it is necessary to characterize the toxicity of the fuels and the types of exposures that may occur during their distribution and use. This is especially true in view of the current concerns for and the laws regulating the biological effects of new potentially toxic substances, as reflected in the NIOSH Registry of Toxic Effects of Chemical Substances (1976) and the Toxic Substances Control Act (P. L. 94-496, 1976). Since little information exists on the toxicity of complex petroleum products, comparative toxicological evaluations of the synthetic and petroleum fuels are desirable in order to (a) compare the suitability of the alternative fuel, (b) assess the adequacy of existing work on environmental controls, and (c) determine the adequacy of the personnel safety procedures.

This paper describes a series of gavage and inhalation studies conducted to determine the toxicity of one petroleum sample and three shale samples of JP5. Although the primary research emphasized study of the neurobehavioral toxicity of these fuels, considerable work was also done in the areas of pathology, hematology, serology, and neurophysiology.

Methods

Experimentation was divided into gavage and inhalation studies using adult, male, Sprague-Dawley rats (N = 660). Since no prior information existed for any of the fuels used here, the oral dosing studies were conducted before the inhalation exposures in order to obtain information on their lethality, to determine the sites of histopathology, and to establish meaningful dose levels for the neurobehavioral studies. The acute gavage experiments included an $LD_{50/14}$ study; a kidney and liver target organ study of 3 days' duration; a liver target organ study lasting 15 days; and behavioral studies of activity, food and water intake, aggression, and skilled motor performance lasting 7 days. The subacute inhalation experiments included somatosensory evoked potentials (SEPs) and the same behavioral tests used in the gavage studies, and they lasted for about 30 days.

Maintenance of Animals

In the general toxicology studies the rats were housed individually in wire suspension cages, 17.5 × 24 × 17.5 cm. A 12-hour light (6 AM–6PM) and 12-hr dark cycle was maintained. Food and chlorinated water were available *ad libitum*. In the behavioral and SEP studies, rats were housed individually in polycarbonate cages. Each cage measured 48.3 × 26.7 × 44.5 cm, and had a raised wire floor, perforated lid, food dispenser, and calibrated water dispenser. These subjects were maintained on an 10-hour light (7 AM–5 PM) and 14-hour dark cycle. Measured amounts of food and water were available during the dark cycle. Room temperature for all studies was kept at 22°C. Humidity was maintained at 60%–70%.

Fuels

One sample of petroleum-derived JP5 and three different samples of shale-derived JP5 were used in these studies. The petroleum JP5 was refined by Hess St. Croix from a mixture consisting primarily of 55% Iranian and 25% Nigerian crude. The shale crude was derived by the Paraho above-ground retorting process from a shale deposit in Anvil Point, Colorado [4]. Shale from this deposit was shipped at different times to three refineries for processing into JP5 and other products. The refineries were Gary-Western (1974), Exxon (1976), and

Sohio (1978–1979). All samples were chemically similar except the shales, which had higher amounts of normal alkanes, partially hydrogenated polynuclear aromatics, and nitrogen (i.e., Gary-Western and Sohio) [5].

Oral Dosing Procedures and Experimental Design

$LD_{50/14}$ study. For each of the four fuels, six groups of six rats each were given a single oral dose of either fuel or water at doses of 24, 30, 38, 48, or 60 ml/kg of fuel and 60 ml/kg of water (control). These doses represent 0.1 log interval steps (1.25 antilog) from a reference point of 60 ml/kg, the maximum dose possible for the rat. Those rats that survived for 2 weeks were sacrificed with carbon dioxide.

All subjects were necropsied, gross lesions were noted, and tissues were prepared for microscopic examination after being fixed in 10% buffered formalin, embedded in paraffin, cut into 6-micron sections, and stained with hematoxylin and eosin (H&E). The following tissues were examined microscopically: larynx, thyroid, esophagus, lung, heart, thymus, kidney, urinary bladder, testis, epididymis, prostate, seminal vesicle, liver, spleen, pancreas, adrenal, stomach, small intestine, colon, brain, and any other tissue with gross lesions.

3-day target organ study. The effects of JP5 on the liver and kidney were compared in each of the four fuels. Each fuel was administered to a group of 18 rats at a dose of 24 ml/kg. This dose-level was used because it was the lowest dose in the $LD_{50/14}$ study that did not produce death. Two groups of 18 rats each served as water-dosed controls: one group for the Gary-Western and Exxon shale experiment, and the other for the petroleum and Sohio shale. Six rats from each fuel and control group were sacrificed with methoxyflurane at 24, 48, or 72 hours after gavage.

Sections of the liver and kidney were prepared for routine light microscopic examination using H&E stain. Frozen sections of selected formalin-fixed liver and kidneys were cut at 20 microns and then stained with Oil-Red-O (ORO) for neutral lipids. Selected specimens were embedded in methacrylate, sectioned at 3 microns, and stained with H&E.

A complete blood count and serum chemistries were performed on blood taken from the abdominal aorta at necropsy. Blood was stored in a glass tube containing the anticoagulant potassium EDTA. The chemistries measured were blood urea nitrogen (BUN), creatinine, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). Serum was stored at −16°C for a maximum of 6 days and then tested on a Gilford System 3500 automated analyzer using reagents supplied by the manufacturer [5]. Two normal controls, a high control, and a standard for each serum chemistry were run before the test samples; three controls were run after each series.

15-day target organ study. The hepatic toxicity of petroleum and Sohio shale was evaluated using four groups of rats, each consisting of 78 subjects. There were two control groups given water and one group given JP5 for each of the two fuels. Each group received 24 ml/kg. Six rats from each experimental and control group were sacrificed with methoxyflurane at intervals of 3, 6, 9, 12, and 18 hours and at 1, 2, 3, 5, 7, 10, 12, and 15 days after gavage. The procedure for obtaining and preparing the blood and hepatic tissue for analysis was the same as for the 3-day study. SGOT and SGPT were measured to assess liver function.

Behavioral studies. Screening for behavioral evidence of toxicity was conducted with both petroleum and Sohio shale JP5. These studies were conducted at 24 ml/kg, the lowest dose tested in the oral $LD_{50/14}$ study. Both the control and treatment groups contained 6 subjects. The tests in this screen included a general behavioral checklist and measurement of food and water consumption, weight, and overnight home-cage activity. Daily measurements were taken for 5 workdays after gavage. Overnight activity was recorded for each rat by counting the number of times the animal broke a light beam from a photoelectric cell mounted outside the long axis of the polycarbonate cage. The food and water dispensers were mounted on the opposite ends of the long axis, so that the subject had to traverse the cage to eat or drink.

thus breaking the beam. The consumption of food and water and the weight was measured for every animal each morning. The overall behavioral condition of the subjects was assessed daily using the observational profiles derived from Balazs [6] and Loomis [7].

Since petroleum JP5 was available for testing for a much longer time than the Sohio shale products, we conducted more extensive behavioral tests with it than with the shale, including the low-dose studies to be reported in this paper. In the first of these studies, rats were gavaged with 3, 5, or 8 ml/kg of petroleum JP5 or 8 ml/kg of water. Each group contained six subjects. Behavioral evaluations were the same as those described above. This study was followed by an assessment of the effects of petroleum JP5 on skilled motor performance. The subjects were tested on the accelerod [8] every 30 minutes after dosing for 6 hours. Four groups of six rats were dosed at either 1, 3, or 5 ml/kg of petroleum JP5 or 5 ml/kg of water administered in a single oral dose.

Inhalation Exposure and Experimental Design

Generation system. Subjects were exposed for 6 hours per day, 5 days per week for about 30 days to 1100 mg/m^3 petroleum JP5 (reported as decane) or to 1600 mg/m^3 Sohio shale JP5 (reported as nominal total hydrocarbons). Inhalation exposures were conducted in 30-liter Leach chambers constructed of glass and stainless steel and ventilated at approximately 10 liters/minute [9]. Temperature was maintained at 23°C and the relative humidity at 50%. Laboratory-supplied compressed air was dehumidified and filtered through activated charcoal 4X molecular sieve before it entered the exposure system. Control chambers were ventilated with air from the same source used in the vapor generating-system.

The mixed hydrocarbon fuels present several special problems in generating, characterizing, and controlling contaminant vapors. Further limitations are usually forced by the conditions of the occupational environment that the exposure is intended to model. The vapor generation system used for the petroleum JP5 exposures was designed to minimize experimental variability resulting from (a) qualitative changes in the composition of the residual fuel in the vapor-source reservoir from hour to hour during a daily exposure or from day to day over the duration of the study; (b) differences in the relative proportions of the chemical constituents in the liquid and the derived vapor; (c) differences in the composition of the vapor phase, which arise from temperature extremes not present in the occupational environment being modeled.

The petroleum JP5 was pumped from a reservoir of fresh fuel at a previously calibrated constant rate into a test tube from which it was aspirated into a dual fluid aerosol nozzle. The vapor-aerosol mixture was forced through a 0.5-micron membrane filter and immediately diluted with clean, dry air at room temperature of 23°C to achieve the vapor concentration desired in the chamber. The exposure mixture and the control chamber air were piped to the chambers through $^3/_8$-inch vinyl tubing.

Calculation of nominal concentrations for mixed hydrocarbon exposures required a total accounting of mass-losses through the system. Accumulation of the liquid fuel in the aerosolizer and leakage from both the filter holder and the aerosolizer prevented accurate measurements of the amount of fuel consumed from the reservoir but not converted to vapor. In order to follow the course of the exposure, a gas chromatograph (GC) flame ionization detector was modified for use as a total hydrocarbon analyzer by connecting it to a continuous sample input loop from the vapor generator and chamber system. The detector response to pure decane vapor from the exposure system was then calibrated against vapor standards prepared in aluminized mylar bags. The concentration of JP5 vapor in the chamber and sampling system was then reported as the concentration of decane that produced an equivalent detector response during calibration. For the Sohio shale experiments, the concentration was increased to approximately 1600 mg/m^3.

Since operation at this level was beyond the effective generation capacity of the aerosol-vapor system previously described, a new generator was constructed, a countercurrent liquid-air-flow, all glass-teflon system (Fig. 1). Dehumidified, filtered, compressed air flowed

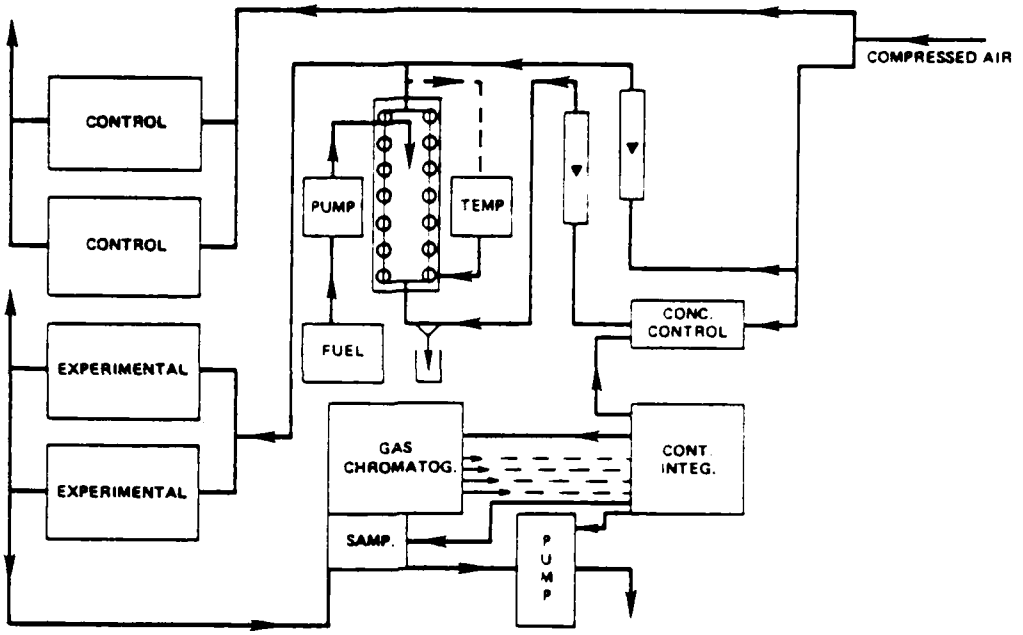

Figure 1. Scheme of inhalation-generation system

through two controlling rotameters to the top and bottom of the generator in a ratio of roughly 4:1. The total dilution and vapor saturation flow was 20 liters per minute.

Fuel pumped from the reservoir trickled down the heated, helically indented inner surface of the inner cylinder; there it vaporized and mixed with air flowing into the bottom. The saturated mixture was diluted to the appropriate concentration at the tee located at the top of the generator, and it was then piped to the Leach chambers in 5/8-inch vinyl tubing. The inner cylinder was heated by a Nichrome wire coil that was wound in the helical indentation in the outer surface of the cylinder. Power to the coil was regulated by a feedback signal from a thermistor detector in the exit tee at the top of the cylinder to a proportional temperature controller. The temperature at the vapor exit was maintained at 50°C. The inner cylinder was surrounded by outer glass housing, which maintained a thermally stable environment for the generator. The conversion of JP5 to vapor in the generator was calculated from mass input for the neat fuel and recovery data for the unvaporized residual. The nominal concentration was computed daily by dividing the weight of fuel vaporized by the total volume of air passing through the system. This concentration was, in effect, a time-weighted average for a day's exposure.

To effectively monitor the environmental conditions during exposures, a semi-quantitative measure of total hydrocarbons as well as quantitative assays for oxygen and carbon dioxide were required. These requirements were met using a GC System controlled by a user-programmable computing integrator. Through a specially designed interface, the integrator automatically controlled GC start-up, sampling pump operation, detector selection, and column-switching valve operation. The recording, integration, calculation, and recycling operations of the GC were controlled by Read-Only-Memory (ROM) functions within the integrator. The automated sampling and column-switching system (Fig. 2) was developed to alternately detect a consolidated hydrocarbon peak by flame ionization and to assay fixed gases by thermal conductivity. In the injection mode, chamber air contained in sample loop 1A was flushed by the helium carrier gas 2A into a series of three columns. Column 3A retained hydrocarbons in 1% Dexsil 300 on 100/200-mesh Gaschrom Q in 18 inches of 1/8-inch-diameter stainless steel. Carbon dioxide was retarded in Column 4A on a 80/100-mesh Poropak Q in a 10 foot × 1/8-inch-diameter stainless steel column. The combined oxygen

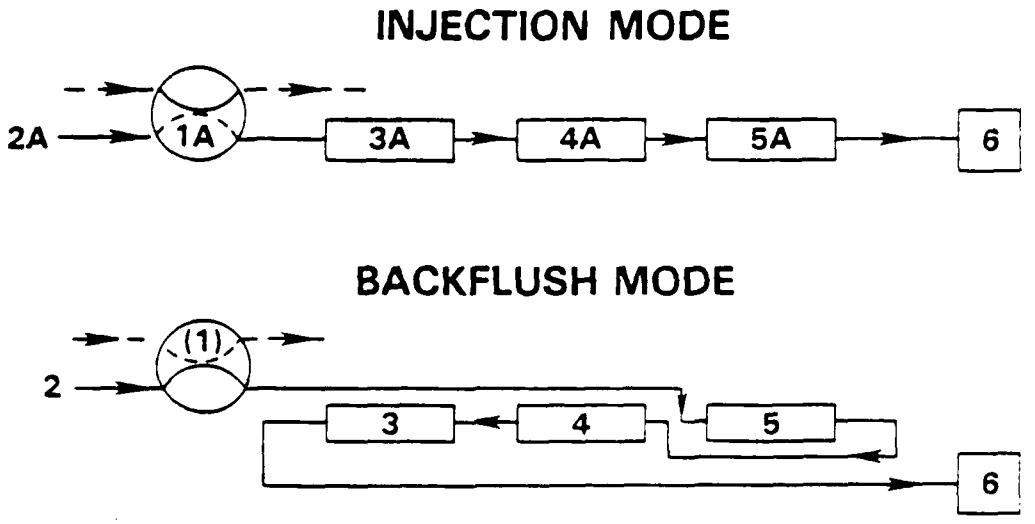

Figure 2. Scheme of automated sampling and switching system

and nitrogen peak passed into a 5 foot × 1/8-inch stainless steel column (5A), packed with 30/60-mesh Type 5A molecular sieve. Before carbon dioxide began to emerge from column 4A, the system was switched to the backflush mode (Fig. 2, lower panel). The flow of carrier gas continued forward through column 5, but reversed in both columns 3 and 4. The hydrocarbons eluted first, followed by carbon dioxide, oxygen, and nitrogen. The column effluent was split between the flame ionization detector and the hot-wire detector in a flow ratio of 2.5:1. The single-channel integrator alternated between the two detectors and automatically adjusted the timing of the backflush valve to maintain appropriate peak shape and elution time. The total carrier flow rate was 35 ml/min; the valve and column ovens were maintained at 80°C; and the hydrocarbons and fixed gases analyses were reported every 12 minutes.

The system was calibrated by five replicate assays of five different concentrations of fixed gases and propane. The calibration standards were prepared in Tedlar bags from various combinations of two cylinders of precision gas standards. The detector response curves were fitted by least squares linear regression to provide the response factors [11].

Behavioral studies. The neurobehavioral toxicity of petroleum-derived JP5 and Sohio shale-derived JP5 was evaluted using the same housing and behavorial parameters as those used in the oral exposures. For each fuel there was one experimental group and one control group, each with six subjects. All animals in each group were collectively exposed in the same Leach chamber.

Somatosensory evoked potential study. The effect of Sohio shale JP5 on the somatosensory system was evaluated using SEPs collected in conjunction with the study on the fuel's neurobehavioral effects, and using the same number of groups and exposure parameters. In order to measure the electrical potential over the somatosensory area of the brain during the 30-day exposure, recording electrodes were surgically implanted. Each subject was medicated with xylazine at 60 mg/kg s.c., anesthetized with sodium pentobarbital (50 mg/kg i.p.), and then positioned in a stereotaxic unit. Stainless steel machine screws (2-56 × 1/8 inch) were implanted into the skull so that the active electrode was located over the right somatosensory cortex (1 mm anterior and 3 mm lateral to bregma), while the reference electrode was placed near the right frontal sinus (3 mm lateral and 5 mm anterior to bregma). Additional attached screws served as support posts for the plug or acted as electric ground. The electrodes were wired to a head plug similar to the one described by Moleno and McIntyre [12]. Dental

acrylic was applied to fasten the plug to the skull. The subjects were allowed 2 weeks to recover before collection of data.

SEPs were collected once a week from the 12 subjects. The stimulating electrodes were Grass E2B subdermal electrodes inserted under the left planter surface, with the cathode approximately 5 mm proximal to the anode. The stimuli were monophasic electrical pulses generated by a Nicolet constant current stimulator at a rate of 3 pulses second, for a 0.2-second duration and sufficient intensity to cause a small twitch in the subject's toes. Each subject was sedated with ketamine (110 mg/kg i.p.) to reduce discomfort from the stimulations and to reduce movement artifacts during the data collection. Each subject was tested at the end of the workweek, thereby allowing 48 hours between the ketamine injection and the next exposure day.

The electroencephalogram was amplified with an impedance, Grass P511 differential preamplifier. The gain was 1000, the input was 2 × 10^{6} ohms, and the bandpass was 0.1 to 10 Hz measured at the ½ amplitude points with a 6-db/octave rolloff. The high frequency cutoff point of 1 kHz was set by a Krohn-hite model 3324R Butterworth filter that had a 24-db/octave rolloff. The data were averaged by a Nicolet 1074 signal-averaging system. The system had an analog-to-digital converter with 9 bits of resolution which passed the data through an RC filter with a 6-db/octave rolloff and a 0.02-msec time constant. The data were digitized at a rate of 12,500 samples per second. Each sweep (epoch) contained 1024 samples and lasted approximately 81 msec. There were 1024 epochs/averaged response. An Ortec model 4710 dual channel stimulator generated pulses that both initially triggered the averager (to establish a baseline) and then, 4 msec later, triggered the constant current stimulator. The entire system was calibrated with a step function 500 microvolts in amplitude and 20 msec in duration. The calibration signal, generated by a Stoelting CA5 triggered pulse-generator, was averaged in the same manner as the data.

The averaged response was transmitted from the Nicolet to a Digital Equipment Corporation (DEC) PDP 11/70 computer. An on-line interactive program was then used to display, analyze, plot, and store the data. Analysis included measuring the latencies and amplitudes of individual peaks and the differences in amplitudes and latencies of the intervals.

Pathology and serology studies. At the conclusion of both inhalation studies, necropsies were performed on all animals. Each subject was anesthetized with methoxyflurane, and blood was drawn for serology (BUN, creatinine, SGOT, and SGPT). The subjects were then whole-body gravity perfused with 4CF-1G dual fixative [13]. After fixation, the tissues were routinely sectioned at 6 micron and stained with H&E. The examined tissues included those for the oral $LD_{50/14}$ study plus nasal cavity, middle ear, bone marrow, skin, salivary gland, preputial gland, skeletal muscle, anus, mesenteric lymph node, and submandibular lymph node. For each subject that was part of the SEP study, the sciatic nerve, lateral saphenous nerve, and brain were also sectioned for neurohistopathology

Analysis of Data

Probit analysis [14] was used to obtain the lethality profiles from the $LD_{50/14}$ studies. In the 3-day study, t-tests were used to analyze the differences between fuel groups and control groups [11]. In the 15-day target organ study, the behavioral gavage studies, and the inhalation studies, the significant treatment effects were tested by a one-way analysis of variance, with Newman-Keuls tests used to identify significant differences between the means of the daily or half-hour test periods [15].

Results

The work presented here was compiled from a number of behavioral and pathology studies using various testing procedures. In general, only the statistically significant and/or meaningful findings (from the point of view of what is known about these materials) are discussed. When specific assessments described in the Methods are not reported, it can be assumed

Table 1

$LD_{50/14}$ FOR RATS FOLLOWING JP5 GAVAGE

	$LD_{50/14}$ (ml/kg)
PETROLEUM	> 60 [a]
SHALE:	
EXXON [b]	> 60
GARY WESTERN	26
SOHIO	39

[a] Highest Dose; Doses ranged from 24 to 60 ml/kg.
[b] Refined by

that the findings were negative. A complete presentation of the $LD_{50/14}$ and 3-day target organ study can be found in a report by Parker *et al.*[5]

Gavage Studies

$LD_{50/14}$ study. The probit values calculated for the four fuels are presented in Table 1. For the Exxon shale and petroleum, the 50% lethality point exceeded 60 ml/kg. Preliminary water-volume studies had shown 6% of body weight to be the limit of stomach capacity; thus, 60 ml/kg was the highest dose that could be given.

At necropsy the majority of gross lesions were similar in all rats that died spontaneously, regardless of fuel type or dose level. The s[illegible]is, lungs, meninges, and epicardium were moderately to severely congested in all rats that died within 24 hours of dosing. In rats that died after 24 hours, the livers were also swollen and mottled with accentuated lobular patterns. In addition, three rats given 48 ml/kg and one given 38 ml/kg Sohio shale had blood in the stomach. Ventral alopecia was the only consistent gross observation for any of the fuels in rats that survived 14 days.

As with the gross examinations, histopathology revealed that lesions in animals that died spontaneously were all similar in nature but varied in severity. Liver lesions consisted of moderate to severe cytoplasmic vacuolization of hepatocytes and hepatocellular degeneration around the portal triads (Fig. 3.). Affected periportal hepatocytes had several sharply bounded cytoplasmic vacuoles that ranged up to 25 microns in diameter. ORO staining of frozen sections showed the entire cytoplasm of the affected hepatocytes to have a mild, diffuse affinity for the stain. Necrosis of individual hepatocytes in all fuel-dosed rats was indicated by pyknosis and karyorrhexis. A few mitotic figures were observed in severely affected livers. In addition to the ORO-positive vacuoles, periportal hepatocytes in rats given Gary-Western shale often had a large number of very small, indistinctly bounded cytoplasmic vacuoles that did not selectively stain with ORO. In less severely affected hepatocytes of this group, small vacuoles were more commonly noted in the periphery of the hepatocyte cytoplasm.

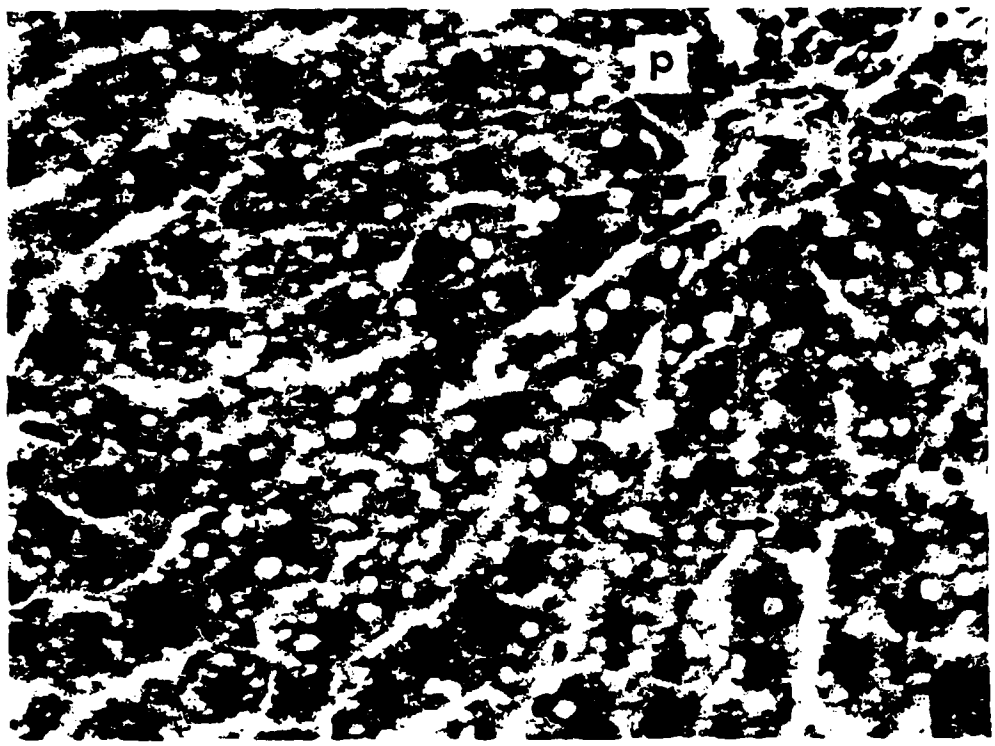

Figure 3. Cytoplasmic vacuolization in periportal hepatocytes in a rat sacrificed 48 hours later after gavage with 24 ml/kg of Exxon shale JP5. Note relatively large, sharply delineated cytoplasmic nuclear debris, and mitotic figure (arrow). P = portal triad. Paraffin embedded, H&E, X559.

The most consistent renal change was the formation of eosinophilic hyaline droplets in the cytoplasm of epithelial cells in the proximal convoluted tubules (Fig. 4). The droplets varied in size and number but tended to be more numerous and larger as the interval between dosing and death increased. In severely affected cells, the cytoplasm was filled with droplets. These droplets were seen in rats that died as early as 48 hours and as late as 83 hours after dosing, but they were not present in rats that died before 48 hours or that survived 14 days. The renal hyaline formation was inconsistently accompanied by numerous small vacuoles in the cytoplasm at the base of proximal tubule epithelial cells. Many vacuoles stained selectively with ORO in frozen sections.

Vascular congestion of variable severity was evident in a number of organs of rats that died within 24 hours of dosing. Acute inflammation of the nonglandular portion of the stomach was seen in three rats given 60 ml/kg Exxon shale and in five rats given either 60 ml/kg or 48 ml/kg Sohio shale. Other gastric lesions consisted of neutrophilic infiltration and vesicle formation in the deep layers of the stratified squamous epithelium. The underlying stratum germanitivum was intact, but small aggregations of neutrophils were seen in the submucosa of the vesicle foci.

3-day target organ study. Gross and microscopic changes were similar to those seen in rats that died at the same time within the first 72 hours of the $LD_{50/14}$ study, with the exception that congestion was not present in the sacrificed rats. Vacuolization of periportal hepatocytes was noted in an occasional rat sacrificed on day 2. The vacuolar change produced by Gary-Western shale was different in that a multitude of fine vacuoles accompanied the larger cytoplasmic vacuolization. This change was more severe in rats given the three shales than in those given petroleum JP5, and it was still present on day 3 in all but two of the Gary-Western group. The conclusion that microscopic changes in vacuolization had occurred was

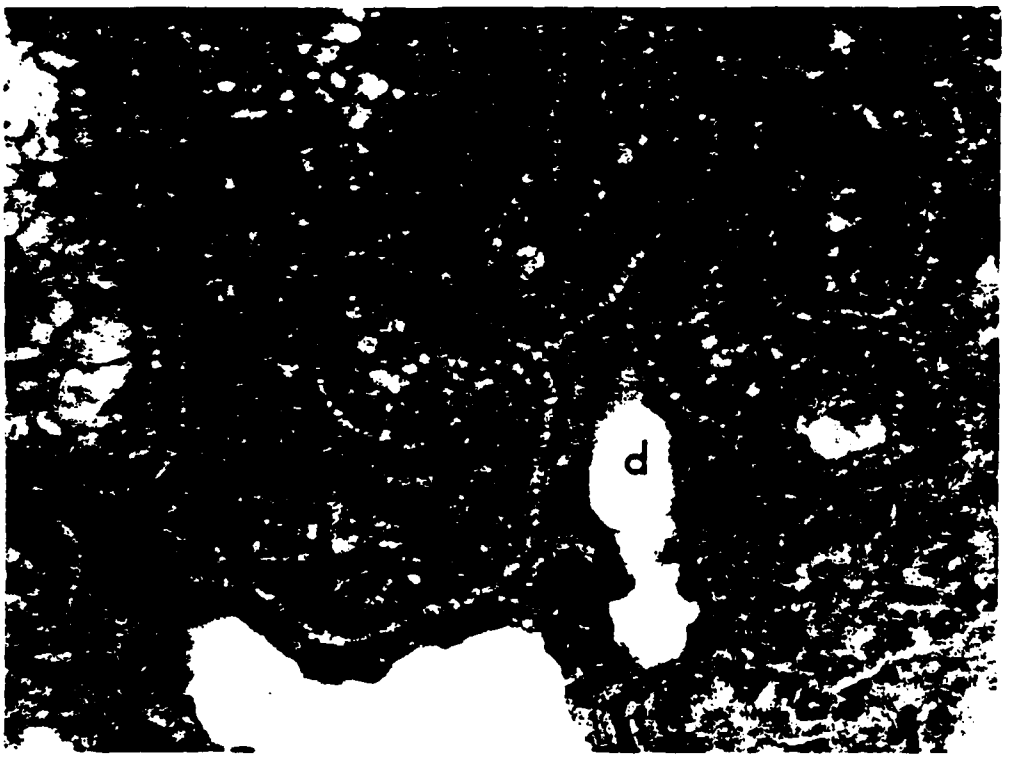

Figure 4. Cytoplasmic hyaline droplets and basel vacuoles in the proximal distal convoluted tubules of a rat sacrificed 48 hours after gavage with 24 ml/kg Gary Western shale JP5. Note sparing of distal tubule (d). Methacrylate embedded, H&E, X996.

supported by the increased levels of SGOT and SGPT (Table 2). Mitotic figures were noted in all livers of treated rats sacrificed on days 2 and 3.

Cytoplasmic droplets were noted in the proximal convoluted tubules of the kidneys of all fuel-dosed rats, but they were larger and more numerous in those rats sacrificed on days 2 and 3. Small vacuoles were noted at the base of some affected epithelial cells. Droplets and vacuoles were much more evident and distinct in kidney specimens embedded in methacrylate and sectioned at 3 microns before staining with H&E. The presence of hyaline droplets correlated with a concurrent rise in serum creatinine and BUN levels (Table 2).

15-day target organ study. Significant increases in SGOT and SGPT levels were found in both the petroleum-exposed and Sohio-shale-exposed rats. Both groups showed significant SGOT effects from 9 hours through day 2 after gavage (Fig. 5). SGPT levels were significantly higher than controls at 6 hours for the petroleum and 9 hours for the Sohio groups (Fig. 6). These levels remained significantly higher through day 2.

Gross pathology revealed swollen livers with extensive surface mottling in rats sacrificed at 1 to 3 days after gavage with both fuels. No congestion in the lungs or epicardium was seen in any treatment group. Examination revealed that the stomach contained up to 50% of the treatment dose 3 hours after intubation; the stomach was completely empty by 6 hours.

Microsopic liver lesions appeared as early as 12 and 18 hours after treatment in the Sohio-shale gavaged and petroleum-gavaged rats, respectively. These lesions consisted of multiple small cytoplasmic vacuoles (ranging from 0.5 to 3.0 microns in diameter) within periportal hepatocytes. ORO staining of frozen liver sections demonstrated lipid material within these vacuoles. This cytoplasmic vacuolization incresed in size (up to 5.0 microns in diameter) and number at subsequent intervals of sacrifice. At the same time, increased numbers of

Table 2

Serum Chemistries

Blood urea nitrogen (mg/dl)

	Day 1		Day 2		Day 3	
	Dosed[a]	Control[b]	Dosed[a]	Control[b]	Dosed[a]	Control[b]
Petroleum	22 ±2	23 ±1	23 ±1	26 ±2	27 ±3	28 ±1
Sohio Shale	18 ±1[d]	21 ±1	27 ±2[d]	21 ±1	25 ±1[d]	24 ±1
G. W. Shale[e]	30 ±2	27 ±1	35 ±4	25 ±1	34 ±6	26 ±1
Exxon Shale	22 ±1[c]	27 ±1	22 ±1	25 ±1	21 ±1[c]	26 ±1

Creatinine (mg/dl)

	Day 1		Day 2		Day 3	
	Dosed[a]	Control[b]	Dosed[a]	Control[b]	Dosed[a]	Control[b]
Petroleum	1.2 ±0.03[c]	0.7 ±0.03	1.2 ±0.05[c]	0.6 ±0.03	0.9 ±0.03[c]	0.7 ±0.02
Sohio Shale	1.6 ±0.15[c]	0.9 ±0.02	1.8 ±0.09[c]	0.8 ±0.02	1.1 ±0.14	0.8 ±0.02
G. W. Shale[e]	1.8 ±0.29[d]	0.8 ±0.06	2.1 ±0.32[c]	0.8 ±0.02	1.9 ±0.11[d]	0.8 ±0.02
Exxon Shale	1.2 ±0.03[c]	0.8 ±0.06	1.2 ±0.07[c]	0.8 ±0.02	1.2 ±0.07[c]	0.8 ±0.02

Glutamic oxaloacetic transaminase (IU/l)

	Day 1		Day 2		Day 3	
	Dosed[a]	Control[b]	Dosed[a]	Control[b]	Dosed[a]	Control[b]
Petroleum	132 ± 9[c]	58 ±2	88 ± 4[c]	45 ±1	140 ±28[d]	76 ± [illegible]
Sohio Shale	170 ±12[c]	77 ±4	132 ± 9[c]	77 ±2	108 ±13[d]	74 ± 4
G. W. Shale[e]	214 ±19[c]	83 ±4	182 ±16[c]	79 ±5	151 ±11[c]	74 ±10
Exxon Shale	71 ±2	83 ±4	74 ± 5	79 ±5	113 ±17	74 ±10

Glutamic pyruvic transaminase (IU/l)

	Day 1		Day 2		Day 3	
	Dosed[a]	Control[b]	Dosed[a]	Control[b]	Dosed[a]	Control[b]
Petroleum	76 ±6[c]	19 ±2	59 ±4[c]	19 ±2	47 ±12	21 ±1
Sohio Shale	108 ±8[c]	16 ±1	60 ±3[c]	17 ±1	21 ± 3	13 ±1
G. W. Shale[e]	52 ±6[c]	17 ±1	75 ±9[c]	18 ±2	61 ± [illegible][c]	18 ±1
Exxon Shale	94 ±6[c]	17 ±1	71 ±5[c]	18 ±2	39 ± 6[c]	18 ±1

[a] Mean ± standard error of the mean for six rats/group gavaged on day 0 with 24 ml JP5/kg body weight.

[b] Mean ± standard error of the mean for six rats/group gavaged on day 0 with 24 ml water/kg body weight.

[c] Significant at $p < 0.01$.

[d] Significant at $p < 0.05$.

[e] Gary Western shale.

dividing hepatocytes were evidenced by mitotic figures and binucleated cells and also occasional pyknotic nuclei of degenerated cells. These changes peaked at 2 days post-treatment for the Sohio shale condition and 3 days post-treatment for the petroleum condition. Significant elevations of the serum chemistries preceded visible liver lesions by 3 hours for SGOT and by 6 hours for SGPT (Figs. 5 and 6).

Visible liver damage appeared to be less severe by day 3 for the petroleum-treated rats and by day 4 for the shale-treated rats. There were a smaller number and size of cytoplasmic vacuoles, a reduced number of binucleated cells, and an absence of mitotic figures and pyknotic nuclei. Essentially normal tissue was observed after day 5 in both treatment groups. The type of histological damage was the same for both fuel groups, and the degree of damage varied only with the time of sacrifice after treatment.

Behavioral studies. The effects of a single oral dose of petroleum or Sohio shale JP5 on food consumption, water intake, weight, and overnight activity for the week following gavage are presented in Figures 7 and 8. The petroleum doses of 3, 5, and 8 ml/kg have been combined because no significant differences occurred between any of the treatment groups and because the overall profile depicted here represent the individual group behavior. These figures reflect the difference between the control group and exposure group on each day for each fuel. The Sohio shale dose shown in Figures 7 and 8 is 24 ml/kg; as explained in the Methods section, this was the only dose tested. Before gavage, behavior was the same in

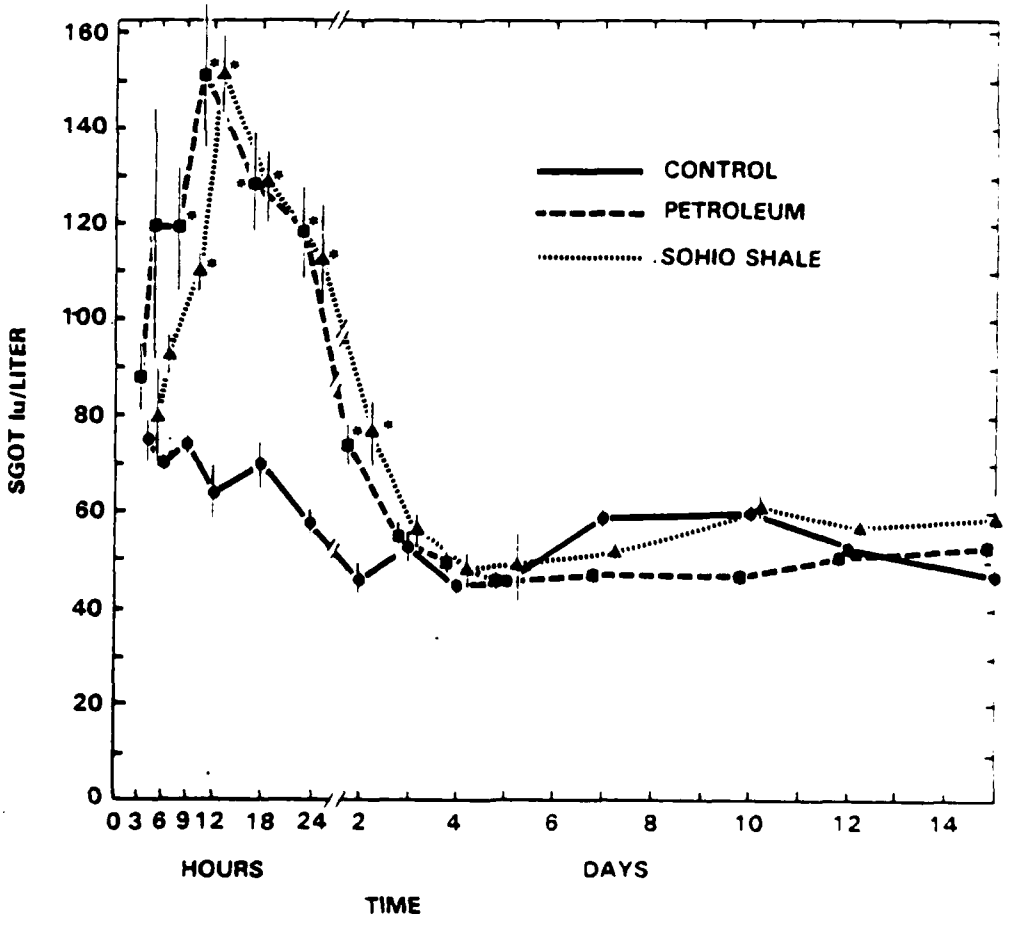

Figure 5. Mean SGOT levels ± standard error for control and fuel treatment groups assessed over 15 days. Asterisks denote significant treatment effects ($p < 0.05$). Note broken time scale between 24 hours and 2 days.

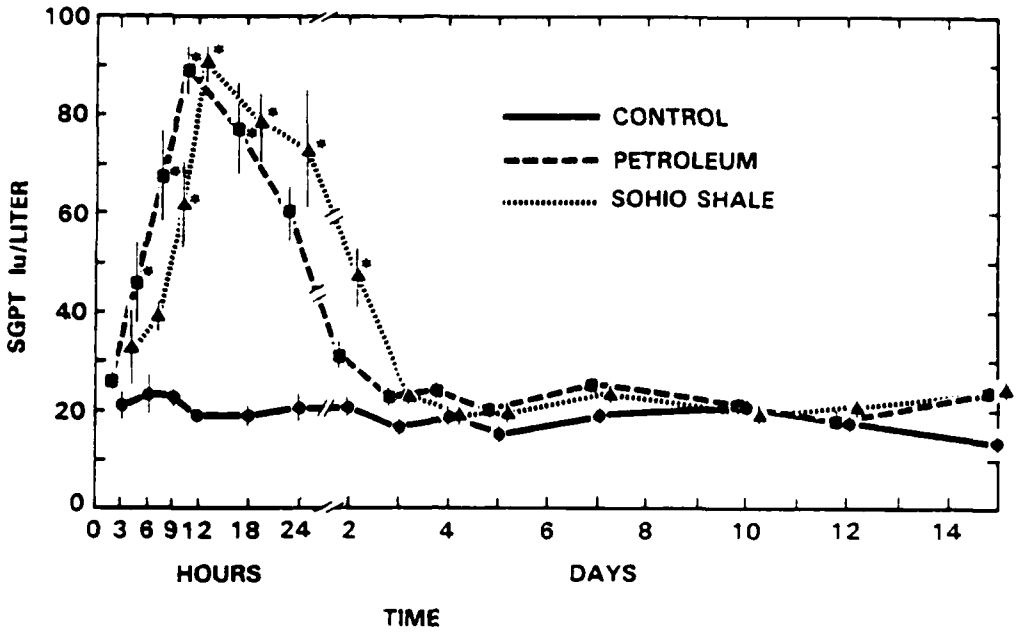

Figure 6. Mean SGPT levels ± standard error for control and fuel-treated groups assessed over 15 days. Asterisks denotes significant treatment effects ($p < 0.05$). Note broken time scale between 24 hours and 2 days.

the control condition and treatment condition for each fuel. The important consideration in these figures is the common direction of effect produced by the fuels rather than the magnitude of effect.

The food intake of both treatment groups was significantly below that of the control group for 3 to 4 days after fuel administration (Fig. 7, left side). Less food was consumed by the exposed subjects on day 2 than on day 1, as indicated by a greater difference between the control and fuel-dosed groups. Since the average food eaten prior to gavage was 23 g for all groups, this meant that on day 2 the petroleum-treated subjects consumed 14 g while the Sohio-dosed subjects ate only 4 g. By day 3, both treatment groups were increasing their food intake, and consumption was normal by day 7.

Both treatment groups showed a significant reduction in water consumption for the first 2 days (Fig. 7, right side). Since water intake averaged 30 ml before exposure, the petroleum subjects drank only half (15 ml) their normal consumption on day 1, while the shale-gavaged subjects' intake decreased to about 33% (20 ml) on day 2. These were the lowest water consumption levels for either group. By day three, the trends reversed when both treatment groups consumed more than controls. This continued until the study's conclusion; the trend was more marked for the shale subjects, peaking on day 4 when these subjects drank almost twice as much as the controls did.

Weight changes for fuel-dosed subjects have been plotted as differences from controls (Fig. 8, left side). Statistical evaluation of these data compared differences in the daily weights of the treated subjects rather than differences between experimental and control subjects, since the weight loss during the first 3 days after treatment produced a significant difference between the two groups and the rate of weight-gain could not compensate for the weight initially lost. The petroleum-dosed subjects lost 30 g or 7% of their body weight by the second day after the acute exposure. On day three, these animals began to gain weight at a rate equal to their pre-exposure baseline, but they never returned to their original weight level with the time limits of this study. Weight loss in the shale-treated animals persisted for 3 days to a maximum of 47 g, or 11% of body weight. A marked, statistically significant increase in body weight occurred in the shale-treated animals on the final day of the study.

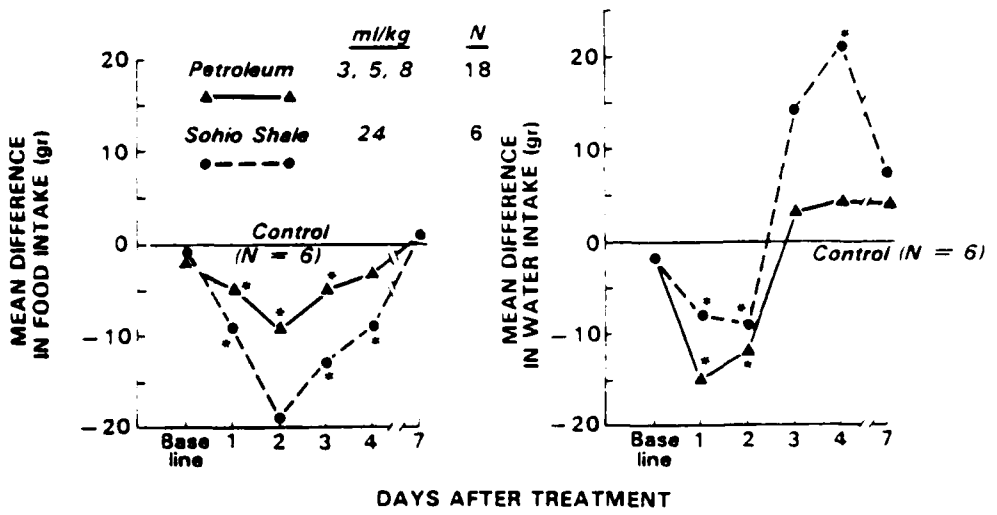

Figure 7. Food and water intake of rats (N = 6/group) gavaged with petroleum or Sohio shale JP5. Profiles are the calculated daily mean differences between control and fuel conditions. Significant effects ($p<0.05$) are shown with an asterisk.

Both fuel-exposed groups experienced a marked and significant increase in home cage activity on the night after gavage (Fig. 8, right side). The average activity before treatment was 274 counts; it increased more than twofold for the petroleum subjects on the evening after treatment and was only slightly less for the shale-treated animals. The activity of the petroleum subjects returned to normal by the second day, whereas the activity of the shale subjects was significantly below normal from day 2 until the conclusion of the study.

Each morning while the subjects were being weighed, their general behavior and condition were assessed. Hair discoloration (a yellow, soiled appearance), oiliness, and matting occurred for both fuels from days 1 to 4, and it progressed upward and forward from the genital area. Alopecia, erythema, and other skin lesions were seen with all but the lowest petroleum dose, starting on day 3 or 4. In the Sohio treatment, two subjects were irritable and/or hyperesthetic on days 3 and 4. Cage movement for two subjects was labored; the subjects appeared to be stiff. On the second day after gavage, pus was noted around the genitals in one Sohio subject and the mouth and/or nose of four subjects was bloody.

In a follow-up study conducted with petroleum JP5 at doses of 1, 3, or 5 ml/kg, home cage activity was measured at 30-minute intervals for 6 hours during the day, a time when rodents usually sleep (Fig. 9). A significant increase in daytime activity occurred 2½ to 4½ hours after dosing in the 3-ml/kg group and at 3½ to 4½ hours in the 5-ml/kg group. A significant effect occurred across the 6 hours of testing for these two groups, whereas activity of the 1-ml/kg group was unaffected.

Inhalation Studies

Generation system. The continuous sampling and flame ionization detector system developed for petroleum JP5 contaminant concentration analysis was found to have a nonlinear response to decane vapor standards. The calibration curve was therefore fitted with the quadratic function $Y = 0.000188\,X^2 + 0.2335\,X + 48.9$ where Y is the detector response measured in counts and X is the concentration of decane in mg/m^3 in the calibration bag. The multiple coefficient of determination was 0.992. The average response to petroleum JP5 vapor over 35 days of 6-hour exposures was equivalent to 1125 ± 86.4 mg/m^3 of decane. The within-day coefficent of variation was 3.1%.

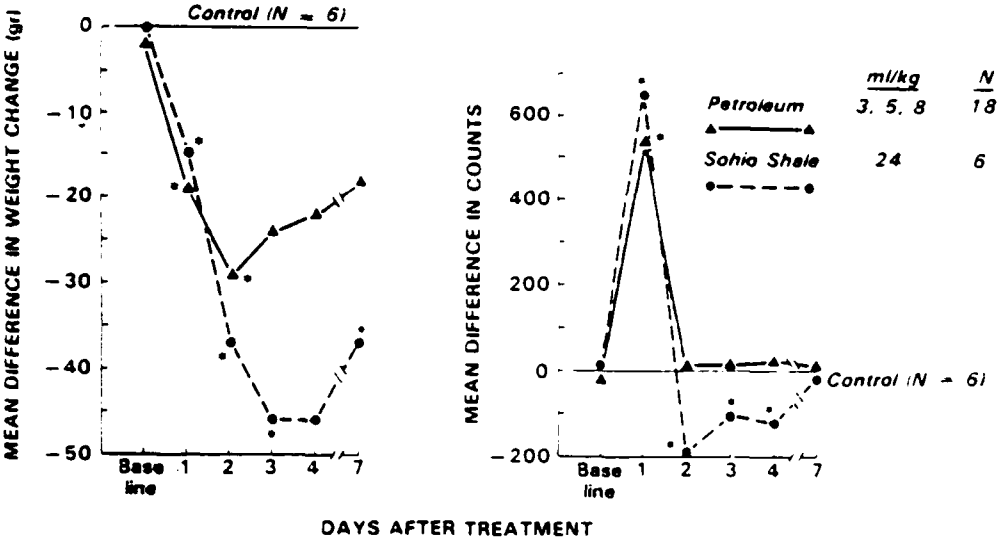

Figure 8. Weight changes and overnight activity of rats (N = 6/group) gavaged with petroleum and Sohio shale JP5. Profiles are the calculated daily mean differences between the control and fuel conditions. Significant effects ($p<0.05$) are shown with asterisk.

In the Sohio shale exposures, the nominal daily concentration averaged 1635 ± 123 mg/m^3, which represents the actual mass loss of JP5 as vapor during a 6-hour exposure. Detector responses to calibration concentrations of propane, oxygen, and carbon dioxide were linear with intercepts at the origin. The variations of shale hydrocarbon concentration as well as those for oxygen and carbon dioxide were quantified during the exposure by direct response factor ratios. The within-day coefficent of variation for the hydrocarbons was 10.8%, while oxygen was maintained in the range of 19% to 21% and carbon dioxide in the range of 0.2% to 0.4%.

Behavioral studies. The only significant behavioral effect was the increased water consumption after exposure to either fuel (Fig. 10). As with the oral data, water intake was plotted as the difference between the control group and treatment group for each fuel. Significant water increases occurred on day 8 for the petroleum group and on day 9 for the shale group. From day 13 to the conclusion of the study, both groups drank more than did controls, with a significant effect across the 30 days of testing.

SEP and pathology studies. No significant effects were produced by Sohio shale on the early components of the SEPs (Fig. 11). The amplitudes and latencies of the P1, N1, and P2 peaks and the intervals between P1-N1 and N1-P2 remained at their baseline levels. Changes in tissue morphology and serum chemistries also did not occur.

Discussion

Although JP5 is a high-flashpoint, kerosene-type aviation turbine fuel that has well-defined military specifications [16], its chemical characteristics are highly dependent on the source of the original crude and the refining process. For example, the crude oil used to make the petroleum JP5 that we tested originated primarily from Iran and Nigeria, and it would have had a slighly different composition if it had come from any other country [17]. On the other hand, the shale JP5's were all derived from the same deposit but had slightly different chemical characteristics because they had been refined by different companies using different processes. Differences in the composition of petroleum products also result from refining differences [18]. As a result, even though the fuels may meet operational specifications,

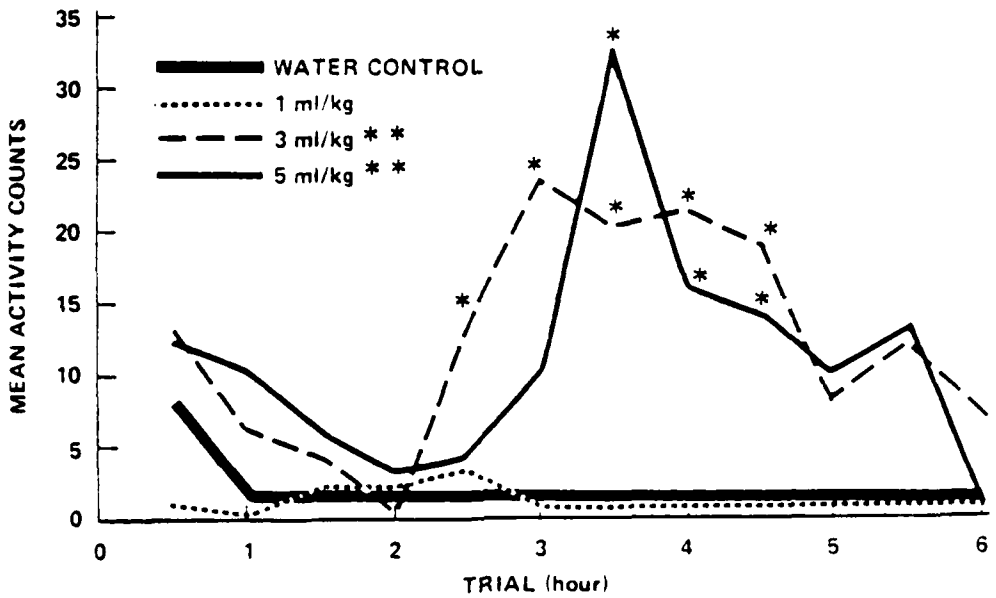

Figure 9. Effects of petroleum JP5 on daytime activity. A single asterisk means activity was significantly different at a 30-minute test interval, and a double asterisk means a significant difference across the 6 hours of testing ($p<0.05$). The groups were equivalent before testing. N = 6/group.

such variability in origin and refining makes it difficult to conduct standardized biological research and to interpret the results of research findings. This difficulty may, in part, explain the paucity of information on the toxicity of these complex mixtures.

Gavage Studies

Nitrogen content appeared to have the greatest influence on the relative toxicity of these fuels. The Sohio and Gary-Western shales had the highest nitrogen concentrations [5] and the lowest $LD_{50/14}$'s. The JP5 from Gary-Western produced the largest changes in BUN and creatinine as well as the most persistent elevations in SGOT and SGPT.

The liver lesions found during the 15-day study occurred 6 hours earlier in the Sohio shale-treated rats than in the petroleum-treated animals. These differences in toxicity apparently arose from differences in the amount of nitrogen that resulted from the various refining processes (since all of the shale crude came from the same source).

The fuels did not cause the same type of distribution of hepatocellular vacuolization as caused by classical hepatotoxins. The four samples of JP5 produced periportal fatty metamorphosis in rats that died or were sacrificed more than 24 hours after dosing, whereas classical hepatotoxins (e.g., carbon tetrachloride) [19 and 20] produce centrilobular fatty metamorphosis and necrosis. Changes in hepatocytes in the periportal region have been associated with potent hepatotoxins that destroy the first cells encountered as the toxin enters the hepatic lobule [21]. Centrilobular pathology is thought to be produced because the central region of the classical hepatic lobule is at the distal end of the blood supply to the lobule and is therefore less richly supplied with oxygen and nutrients [22 and 23]. Although the histological findings suggest that JP5 may have the same mechanism as potent hepatotoxins, the moderate elevation in liver-associated serum enzymes does not support such an estimation of severity.

The primary alteration in the liver was considered to be membrane damage, manifest by the release of cytoplasmic enzymes into the bloodstream and later by accumulation of endogenous lipids in hepatocytes. Lactate dehydrogenase is normally present in the cytoplasm

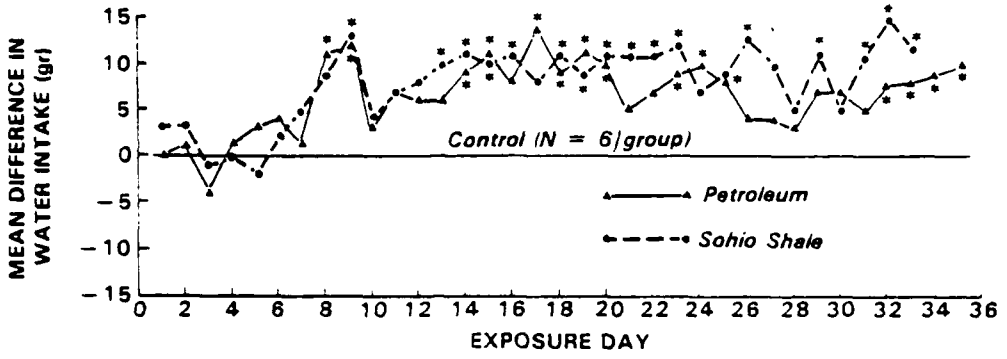

Figure 10. Water intake of rats (N = 6/group) inhalation-exposed to petroleum or Sohio shale JP5 for 5 days/week, 6 hours/day. The profiles are the daily calculated mean difference between control and exposure conditions. Significant effects are shown with an asterisk ($p<0.05$).

of hepatocytes; GOT and GPT are present in the cytoplasm and mitochondria, respectively [24]. Mild hepatocellular injury releases all three cytoplasmic enzymes and severe injury releases mitochondrial GOT and GPT. In the 3-day studies, serum LDH, SGOT and SGPT were substantially above control levels 1 day after dosing, indicating an alteration in cytoplasmic membrane permeability. By contrast, microscopically visible changes in the liver were not apparent until day 2. Microscopically visible alteration consisted of formation of cytoplasmic vacuoles, presumably fatty metamorphosis (thought to result from damage to membrane-associated intracytoplasmic processes), and accumulation of endogenous lipids.

Alterations in membrane integrity may have occurred also in the kidney. Hyaline droplets were present in epithelial cells of the proximal convoluted tubules of the kidneys of all rats dosed in the 3-day study. This change was concurrent with a rise in serum BUN and creatinine, and is consistent with renal impairment. This droplet formation in the proximal convoluted tubules is known to result from an increase in the amount of protein that filters through the glomerulus into the tubule [25], as well as from the impairment of mechanisms by which epithelial cells of the proximal tubule degrade pinocytosed protein [26]. Degradation of pinocytosed protein in the normal epithelial cell depends on fusion of lysosomes with the membrane-bound accumulation of protein in the cytoplasm [27]. In addition to possible loss of membrane function, it is also possible that JP5 damaged the filtration apparatus of the glomerulus in a manner not visible by light microscopy, with the result that an excessive amount of protein was presented to the proximal convoluted tubule.

Clinical signs often seen in humans and farm animals that have accidentally ingested fuel [28–31] were observed in the fuel-dosed rats. For at least 2 days after gavage, the consumption of water and food decreased. The water intake returned to above normal by the third day and the food intake returned to normal by the 7th day. The weight fluctuations closely followed those for food and water. Experimental intoxication of cattle exposed to sublethal doses of sweet crude demonstrated a similar pattern of anorexia and recovery [29]. However, the most serious clinical sequelae in humans and animals, emesis followed by aspiration pneumonia, did not occur in the rats because of their inability to vomit [32]; otherwise, the rats responded clinically like the other species that have been reported.

The hyperactivity observed during the night after dosing with both petroleum and Sohio shale has not been reported in other cases of hydrocarbon ingestion. In fact, humans and farm animals (28–31) are usually depressed following ingestion. Earlier research conducted in this laboratory with petroleum DFM had shown that overnight activity was at or below baseline levels for 1 week after gavage [33]. Home-cage activity after petroleum exposure was therefore measured during the daytime instead of the evening in order to determine whether dormant-cycle activity would be affected and to define the level and temporal pattern

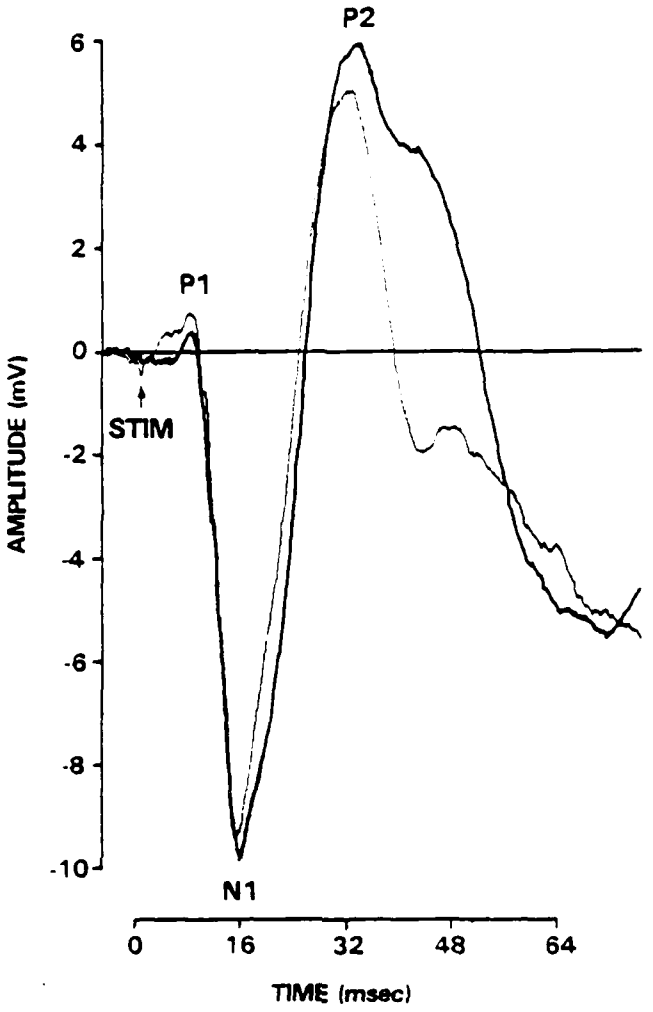

Figure 11. Composite SEPs for the treatment group inhalation-exposed to Sohio shale JP5 for 5 days/week, 6 hours/day for 33 days. The heavier line represents SEPs before exposure and the lighter line, the end of exposure. SEPs were constructed by averaging the responses for responses for the six subjects in the group. Stimulations were evoked at the left plantar surface (arrow) at a rate of 2 per second, 1024 responses per average, and recorded over the right primary somatosensory area. No significant differences were seen between the pre- and post-exposure SEPs in either amplitudes or latencies of the three major peaks or the intervals between the peaks.

of the hyperactivity. This daytime assessment showed that the hyperactivity began at about 2 hours after gavage and ended 4 hours later. A test of skilled motor performance was then conducted to see if these activity changes were due to neuromotor dysfunctions. Motor function on the accelerod [8] was not affected. Thus, since the 15-day study showed that the time course of fuel-evacuation from the stomach was the same as the increase in activity, i.e., 6 hours, it was concluded that the hyperactivity was due to gastric irritation resulting from the rats' inability to vomit, and not to neurobehavioral toxicity.

In other related studies with petroleum JP5 rats were observed to be more aggressive after dosing. Several cases of self-mutilation occurred and frequent instances of hypersensitivity to touch were noted from days 1 to 4. To objectively assess these behaviors, ten petroleum-

dosed and ten water-dosed rats were assessed, using a test that measured shock-elicited aggression [34]. In 6 days of testing, no changes in aggression were observed, and the previously observed aggressivity and hyperesthesia were not present. As with the hyperactivity, changes in aggressivity may be due to gastric irritation, and they do not persist.

Inhalation Studies

It has been generally assumed, although not tested [35], that exposure to petroleum products does not cause serious, long-term adverse effects [36]. But recent reports of polyneuropathies developing in workers occupationally exposed to petroleum jet fuels [37–39] have suggested that the earlier assumptions may be incorrect. Because these recent studies reported cases of neurasthenia and hyperesthesia, our inhalation experiments included tests of motor performance and sensory nerve conduction. The fact that neither fuel significantly affected the SEPs or performances on the accelerod suggests that either JP5 is not neurotoxic or its neurobehavioral toxicity will become manifest only if (a) the exposure durations are lengthened, (b) the concentrations are increased, or (c) the hydrocarbon standard used for reference is less restrictive (the standards used here sampled only low-boiling hydrocarbons). The lack of neurotoxicity here may be due to its relatively high concentration of complex hydrocarbons. Rowe *et al.* [29] suggested that signs of neurotoxicity in cattle are due to the systemic distribution of low-molecular-weight hydrocarbons. Long-chain hydrocarbons may not be able to cross the blood-brain barrier and induce neural damage. However, it is most likely that persistent neurobehavioral effects were not detected in the present studies because of the short duration of the exposure to JP5. This is consistent with the observation of Knave *et al.* [37–39] that polyneuropathies occur only after prolonged exposure to jet propulsion fuel.

The only significant change observed during the inhalation studies was the polydipsia, which was manifest on about the 8th day and lasted for the entire exposure. Although the histopathology and serology did not indicate renal damage, polydipsia is known to be a common, early clinical sign of renal disease [40]. Frank pathology may have occurred if these studies had lasted longer than 30 days. Confirmation for this hypothesis was found in another petroleum JP5 inhalation study conducted in our lab that lasted 60 days. It produced polydipsia and renal histopathology and reduced the clearance of phenol sulfon phthalein [33]. The occurrence of renal toxicity in both the gavage and inhalation studies suggests that the effects of JP5 on the kidney are independent of the crude's origin, the refinery process, and the route of administration.

Summary

Gavage Studies

1. The $LD_{50/14}$ for rats was 26 ml/kg for Gary-Western shale, 39 ml/kg for Sohio shale, and greater than 60 ml/kg for Exxon shale and petroleum JP5.
2. Significant hepatic periportal fatty degeneration and renal eosinophilic hyaline droplets were observed for all fuels.
3. Multiple hepatic cytoplasmic vacuoles were detected as early as 6 hours after both petroleum and Sohio shale JP5 exposures and were undetectable after 96 hours.
4. Weight and consumption of food and water were reduced for 2 to 3 days after administration of petroleum or Sohio shale JP5.
5. Activity markedly increased between 2.5 and 6 hours after dosing for both petroleum and Sohio shale JP5.

Inhalation Studies

6. Water consumption increased after 8 days of exposure to petroleum or Sohio shale and remained elevated for the duration of the 30-day studies.

7. No significant effects on tissue morphology or hepatic and renal serum chemistries were observed after exposure to petroleum or Sohio shale JP5.
8. Peak amplitudes or latencies for the SEPs did not significantly change during the 30-day exposure to Sohio shale JP5.

Acknowledgements

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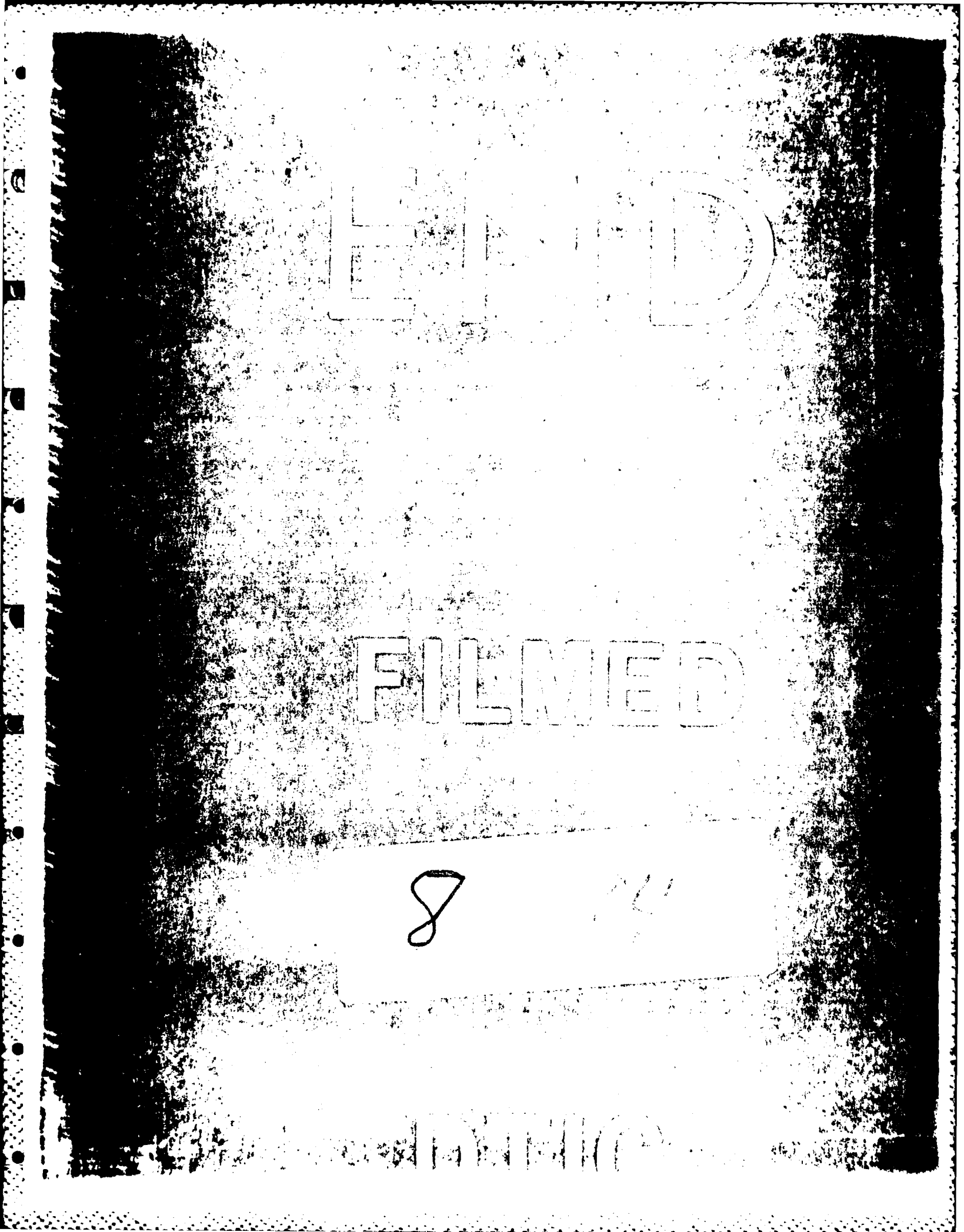
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